

Technical field

The present invention relates to a novel use of a positively charged support. More closely, the invention relates to a sample loading support paper or membrane for loading samples onto an electrophoretic IPG (immobilised pH gradient) gel. The support is provided with positively charged groups and is used to load samples from the cathode side of the IPG gel or strip.

Background

One type of widely used electrophoresis is isoelectric focussing, wherein substances, such as proteins, are separated according to their pI-value. For isoelectric focussing, sample loading has traditionally been performed by cup loading by placing a cup on the gel and letting a sample pass through the cup into the gel. The cup is positioned on the gel for the whole electrophoresis run.

Alternatively for dried gels, the sample may be mixed with electrophoresis buffer and used as a rehydration solution to rehydrate the dried gel, such as Immobiline DryStrip™ gels.

More recently, sample application paper in the form of conventional filter paper, has been placed between the electrode and the electrophoresis gel to load a sample into an electrophoretic gel. This functions satisfactorily for sample application from the anode side of the gel. However, this approach does not work when using acidic pH intervals. As an alternative, rehydration loading can be used in these pH intervals.

However, rehydration loading is not possible with swollen gels, such as pre-swollen RTG (ready-to-go) strips. Thus, these kind of gels need an alternative loading, especially for application of large samples which is very difficult today.

Supports provided with positively charged groups are known within prior art. For example, US 3 714 010 describes anion exchange membranes from cellulosic sheet materials such as cellophane, parchment paper or kraft paper. The membrane is especially suited for use in the electrodialytic purification of saline water.

US 4 080 171 describes a method for analysis of trace components in a liquid, which comprises filtering said liquid through a filter paper having at least one anion exchange.

US 5 151 189 describes a cationic charge modified microporous membrane. This membrane can be used in various applications such as filtration of fluids and macromolecular transfer from electrophoresis gels. The transfer process, also known as "blotting", is defined herein as the steps involved in physically moving biomolecules from a gel matrix to a microporous membrane onto which they become immobilised.

The most common prior use of anion exchange supports within prior art has been the use of anion exchange paper for chromatography purposes. Examples of this are DEAE- cellulose paper and aminoethyl-cellulose paper.

According to our knowledge there is no prior art describing IPG electrophoresis sample loading with positively charged support.

Summary of the invention

The present invention provides an alternative way to load samples onto electrophoretic IPG gels. The invention enables sample loading from the cathode side of the IPG gel or strip. According to the invention sample is applied to an acidic interval IPG gel or strip, such as a RTG (ready-to-go) strip. This novel application enables sample loading in preparative amounts of protein.

The above was achieved according to the invention by providing use of a positively charged support for sample application from the cathode side of the gel. Thus, the invention provides a new method of using a positively charged support.

Thus, in a first aspect, the invention relates to use of a hydrophilic support derivatised with positively charged groups, for sample application to electrophoretic gels, such as IPG (Immobilised pH gradient) gels. According to the invention the application is performed from the cathode side of the electrophoretic gel.

The support is preferably made of regenerated cellulose, dextran, agarose, polyvinylalcohol, polyether sulfone, polysulfone, cellulose acetate, polyurethane, polyamide, nylon or other types of membranes and composite membranes.

Preferably, the positively charged groups are cation groups. The degree of substitution with cation groups on the support may not cause adsorption of substances present in the sample, such as proteins, to the support.

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Preferably, the cation groups are quaternary groups, such as QAE or Q groups, or DEAE.

In the currently best mode, a preferred support is made of regenerated cellulose substituted with a low degree of quaternary groups, preferably Q-groups.

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In a preferred embodiment, the IPG gel is an acidic interval (such as pH 3.5-5) IPG gel or strip. One type of preferred IPG strips are RTG (ready-to-go) strips. RTG-strips are pre-swollen gels available in different pH-intervals.

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The sample applicator according to the invention may be used in analytical as well as preparative amounts, a preferred use is for application of samples in preparative amounts.

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The sample applicator may be used for application of samples to IPG gels per se or used for 2D gels, wherein the first dimension is isoelectric focussing and the second dimension is according to molecular weight.

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In a second aspect, the invention relates to a kit comprising a positively charged sample application support according to the above and an IPG gel, preferably a pre-swollen RTG strip, and more preferably an acidic interval RTG-strip, such as pH 3.5-5, pH 3.5-4.5 or pH 4-5.

In a third aspect, the invention relates to a sample applicator for IPG electrophoresis comprising regenerated cellulose derivatised with cation groups, preferably Q-groups.

Detailed description of the invention

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The present invention provides novel use of a positively charged support, namely as a sample applicator in IPG electrophoresis. According to the invention the support is a hydrophilic support with high water absorbing capacity. Preferably the support can hold a large sample volume, such as 1 ml sample. The amount of sample added to the support is usually from 50 µl

– 10000 µl in a concentration of up to 10 mg/ml. The support must be substantially inert to the substances, such as proteins, present in the sample.

5 The support is made of any material with high water absorbing capacity, such as, but not limited to, regenerated cellulose, dextran, agarose, polyvinylalcohol, polyether sulfone, polysulfone, cellulose acetate, polyurethane, polyamide, nylon or other types of membranes and composite membranes.

10 According to the present invention, the support is substituted with positively charged cation groups, such as DEAE (diethylaminoethyl) or quaternary groups (for example Q (quaternary ammonium) or QAE (quaternary aminoethyl) groups) to give the paper a positive charge and anionic exchange character. This support can be used for application of samples from the cathode side of the gel.

15 The technique for derivatising the support or paper is known per se and can be found, for example, in "Membrane chromatography: Preparation and Applications to Protein Separation" Xianfang Zeng, Eli Ruckenstein; Biotechnol. Prog. 1999, 15, 1003-1019.

20 A preferred support is made of regenerated cellulose (paper) substituted with a low degree of quaternary ammonium groups, preferably Q-groups.

25 The thickness of the support depends on the support material. For regenerated cellulose (paper) the thickness is preferably 3-4 mm. The dimensions of the support are determined by the size of the gel and the sample amount.

The sample loading support according to the invention may be used in association with any swollen electrophoretic gel, preferably an IPG gel.

30 The sample is added to the support and thereafter it is placed between the cathode and the electrophoresis gel. At one end the support is in contact with the cathode and at the other end in contact with the cathode side of the gel. The running conditions are the same as for any IPG run or 2D electrophoresis run.

When using conventional cup loading, there are often disturbances in the first 15% of the gradient due to the presence of the cup. For short IPG strips this may be a very significant portion of the gel. With the present invention this problem is avoided.

- 5 The sample may be loaded in analytical or preparative amounts. The sample may be a biological sample or any other sample.

The present invention is especially suited for application of large sample amounts up to 1 ml and up to 10 mg/ml and is therefore very useful for preparative runs of large amounts of sample, preferably large amounts of protein.

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EXPERIMENTAL PART

Below the invention will be described by a non-limiting example. Although DEAE-groups are mentioned as an exemplifying group, the skilled person could easily employ for example Q-groups instead.

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Cleangel Electrode strip was used as a paper bridge for sample application. This matrix is a paper made of pure cotton linters. Thus, the alpha cellulose content exceeds 98% and the remaining percentage consists of beta and gamma cellulose.

20 Table 1.

Chemicals	Supplier	Article no.	Batch no.
Clean Gel Electrode Strips	Schleicher & Schüll	18-1035-33	
<i>Synthesis of ion-exchanger paper</i>			
Diethyl amino ethyl-chloride, 65%	Amersham Biosciences		
Sodiumhydroxide 0,01 M	Merck		
Ethanol 99,5%	Kemetyl		
<i>Iso-electric focusing</i>			
Immobiline DryStrip pH 3-5.6	Amersham Biosciences		
IPG buffer 3-5.6			
Urea 6M			
Thiourea 2M			
Chaps 2%			
DeStreak	Amersham Biosciences		
IPGbuffer 3-10			
DTT 40 mM			

Synthesis of ion exchangers

The cellulose paper was cut into pieces of approximately 1x2,8 cm and placed into a 20 ml glass vial. The paper pieces were soaked in distilled water (15 ml) and pH was adjusted to >10 with sodium hydroxide. The reaction was started by addition of diethylaminoethylchloride (DEAE, see Table 2 below). The reaction vessels were placed at a shaking table and the reaction proceeded for approximately 19 hours (at room temperature) before neutralization with acid (1 M hydrochloric acid or 1M acetic acid). The papers were washed repeatedly with acid (120 ml), ethanol (720 ml) and water (300 ml) both ultrasonically and on a glass filter. The paper pieces were dried under vacuum over night.

Table 2. Amount DEAE-chloride in relation to cellulose paper

ID	Paper	DEAE-chloride	DEAE-chloride	NaOH
	g	w/w%*	mmol	mmol
U1275004:01	1,51	4	0,287	0,750
U1275004:02	1,56	21	1,44	3,75
U1275004:03	1,56	44	2,86	7,50
U1275004:04	1,52	65	4,31	11,25
U1275006:01	1,1244	1	0,058	0,012
U1275006:02	1,068	0,6	0,029	0,006
U1275006:03	1,1903	0,3	0,014	0,003
U1275006:04	1,1429	0,1	0,007	0,0015

*w/w% DEAE-chloride in relation to the weight of paper.

Isoelectric focusing in Immobiline DryStrip 3-5.6

Immobiline DryStrip pH 3-5.6 were run according to the instructions of the manufacturer. The strips were rehydrated with 0.5 % IPG buffer 3-5.6, 6 M urea , 2 M thiourea, 2 % chaps and DeStreak.

The following sample was soaked into each paper bridge: 220 µl / strip of : 20 µl E. coli extract + 200 µl sample buffer 0.5 % IPG buffer 3-10, 40 mM DTT, 6 M urea , 2 M thiourea, 2 % chaps.

Table 3. Results from iso-electric focusing

Paper ID	Experiment	Added conc of DEAE	Measured conc of DEAE*	Results in IPG strip pH 3-5,6	
				Anode	Cathode
U1275004:1	040518	4	0,06	--	---
U1275004:2	040518	21	0,23	--	---
U1275004:3	040518	44	0,34	---	---
U1275004:4	040518	65	0,36	-	---
U1275006:1	040602	1	0,013	-	++
U1275006:2	040602	0.6	a	0	++
U1275006:3	040602	0.3	a	0	+
U1275006:4	040602	0.1	a	0	+
Original paper	040518/040602	0	0	0	0

*Concentration measured by elemental analysis of CHN at Mikrokemi AB, Uppsala, SE

Key to results	
0	Results as in original paper
+	Better than original paper
++	Best paper tested
---	No visible bands
a	Too low conc. for analysis method

The original paper (0, see Table 2) gave about the same results when sample were applied at the anode or by in gel rehydration loading. Original paper applied at the cathode gave only weak acidic protein band.

The results indicate that the substitution degree of DEAE groups cannot be too high. For the four first mentioned papers the substitution degree was far too high and the paper was acting as a strong ion exchanger thus binding the proteins. This was indicated by the hard adsorption of marker stain Bromophenolblue to the paper. The stain did not / slowly migrated out of the paper during the electrophoresis.

CLAIMS

1. Use of a hydrophilic support derivatised with positively charged groups, for sample application to an IPG (immobilised pH gradient) gel.
2. Use according to claim 1, wherein the IPG gel is an acidic interval IPG gel.
3. Use according to claim 1 or 2, wherein the support is made of regenerated cellulose, dextran, agarose, polyvinylalcohol, polyether sulfone, polysulfone, cellulose acetate, polyurethane, polyamide, nylon or other types of membranes and composite membranes.
4. Use according to claim 1, 2 or 3, wherein the positively charged groups are cation groups.
5. Use according to claim 4, wherein the cation groups are quaternary groups.
6. Use according to claim 5, wherein the quaternary groups are QAE or Q groups.
7. Use according to claim 4, wherein the cation groups are DEAE- groups.
8. Use according to any of the above claims, wherein the IPG gel is a pre-swollen RTG (ready-to-go) gel.
9. Use according to one or more of the above claims, wherein the support is made of regenerated cellulose derivatised with quaternary groups.
10. Use according to claim 9, wherein the quaternary groups are Q-groups.
11. Use according to one or more of the above claims, wherein the sample is applied in preparative amounts.
12. Use according to one or more of the above claims, as a first step in 2D electrophoresis.
13. Kit comprising a positively charged sample application support according to any of the above claims and an IPG gel or strip.

14. Kit according to claim 13, wherein the IPG gel is a RTG-gel.

15. Kit according to claim 14, wherein the RTG-gel is an acidic interval RTG-gel.

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16. Kit according to claim 15, wherein the acidic interval is pH 3.5-5.

17. Kit according to one or more of the claims 13-16, wherein the support is made of
regenerated cellulose derivatised with Q-groups.

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18. Sample applicator for IPG electrophoresis, comprising regenerated cellulose derivatised with
cation groups.

19. Sample applicator according to claim 18, comprising regenerated cellulose derivatised with
Q-groups.

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/SE 2004/001872

A. CLASSIFICATION OF SUBJECT MATTER		
IPC7: G01N 27/447, B01D 57/02 According to International Patent Classification (IPC) or to both national classification and IPC		
B. FIELDS SEARCHED		
Minimum documentation searched (classification system followed by classification symbols)		
IPC7: G01N, B01D		
Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched		
SE,DK,FI,NO classes as above		
Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)		
WPI DATA, EPO-INTERNAL, PAJ, COMPENDEX		
C. DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 9627787 A1 (GENETIC BIOSYSTEMS, INC.), 12 Sept 1996 (12.09.1996), page 5, line 1 - line 18; page 11, line 24 - page 12, line 9, figure 5b --	1-19
X	WO 9912025 A1 (MEDICAL RESEARCH COUNCIL), 11 March 1999 (11.03.1999), page 2, line 9 - page 3, line 2; page 7, line 19 - line 20 --	1-19
X	WO 9800706 A1 (GENETIC BIOSYSTEMS, INC.), 8 January 1998 (08.01.1998), page 6, line 1 - line 19; page 15, line 1 - line 14; page 26 - page 27 --	1-19
<input checked="" type="checkbox"/> Further documents are listed in the continuation of Box C. <input checked="" type="checkbox"/> See patent family annex.		
* Special categories of cited documents: "A" document defining the general state of the art which is not considered to be of particular relevance "E" earlier application or patent but published on or after the international filing date "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) "O" document referring to an oral disclosure, use, exhibition or other means "P" document published prior to the international filing date but later than the priority date claimed "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention "X" document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone "Y" document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art "&" document member of the same patent family		
Date of the actual completion of the international search 1 March 2005		Date of mailing of the international search report 11-03-2005
Name and mailing address of the ISA/ Swedish Patent Office Box 5055, S-102 42 STOCKHOLM Facsimile No. +46 8 666 02 86		Authorized officer Lars Wallentin/ELY Telephone No. +46 8 782 25 00

INTERNATIONAL SEARCH REPORT

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C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 6376231 B1 (TOMOICHI ENOMOTO ET AL), 23 April 2002 (23.04.2002), column 3, line 20 - line 33; column 10, line 4 - line 25 --	1-19
A	Biotechnol. Prog, Volume 15, 1999, Xianfang Zeng et al, "REVIEW: MEMBRANE CHROMATOGRAPHY: PREPARATION AND APPLICATIONS TO PROTEIN SEPARATION", page 1006, line 2 - page 4, line 10, Table 4 -- -----	1-19

INTERNATIONAL SEARCH REPORT

International application No.

PCT/SE 2004/001872

WO	9627787	A1	12/09/1996	AU	5418996 A	23/09/1996
				EP	0871870 A	21/10/1998
				US	5972188 A	26/10/1999

WO	9912025	A1	11/03/1999	AU	8874398 A	22/03/1999
				GB	9718320 D	00/00/0000

WO	9800706	A1	08/01/1998	AU	3592297 A	21/01/1998
				EP	0979403 A	16/02/2000
				US	5972188 A	26/10/1999

US	6376231	B1	23/04/2002	JP	2000298116 A	24/10/2000
